Claims

1. (Currently amended) A method for detecting ketosteroids, comprising:

reacting a sample with a sulfonhydrazide to form a sulfonhydrazone of a ketosteroid in the sample; and

analyzing the reacted sample by ~~ionization~~ mass spectrometry to detect the ketosteroid by detecting the sulfonhydrazone of the ketosteroid, wherein detection of the sulfonhydrazone indicates presence of the ketosteroid.

2. (Currently amended) The method of claim 1, wherein ~~the ionization~~ analyzing the sample by mass spectrometry comprises ~~an~~ atmospheric pressure ionization. ~~spectroscopy.~~

3. (Currently amended) The method of claim 2, wherein ~~the~~ atmospheric pressure ionization ~~spectroscopy~~ comprises positive ion mode electrospray ionization. ~~spectroscopy.~~

4. (Currently amended) The method of claim 1 further comprising separating the ketosteroid from other components in the sample by liquid chromatography.

5. (Original) The method of claim 4, wherein the liquid chromatography is high performance liquid chromatography (HPLC).

6. (Currently amended) The method of claim 4[[,]] wherein the ketosteroid ~~ketosteriod~~ is reacted with the sulfonhydrazide prior to separating the ketosteroid by liquid chromatography.

7. (Currently amended) The method of claim 5[[,]] wherein separating the ketosteroid from other components in the sample by HPLC comprises reverse phase HPLC using a non-polar stationary phase.

8. (Currently amended) The method of claim 7 wherein reverse phase HPLC is performed using a methanol/water solvent. ~~and a non-polar stationary phase.~~

9. (Currently amended) The method of claim ~~8~~ 7, wherein the non-polar stationary phase is a C18 stationary phase.

10. (Currently amended) The method of claim ~~5~~ 8, wherein HPLC is performed with gradient elution from 20:80 methanol/water to 80:20 methanol/water is used.

11. (Currently amended) The method of claim ~~5~~ 10, wherein gradient elution is performed from 40:60 methanol water to 60:40 methanol water is used.

12. (Original) The method of claim 1 further comprising extracting the ketosteroid from the sample prior to reacting the sample with the sulfonhydrazide to provide a concentrated sample for analysis.

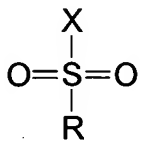
13. (Currently amended) The method of claim 1, wherein the ketosteroid is an estrogen.

14. (Currently amended) The method of claim 13, wherein the ketosteroid is a catechol estrogen.

15. (Currently amended) The method of claim 1, wherein the sulfonhydrazide is *p*-toluenesulfonylhydrazide.

16. (Currently amended) The method of claim 1[[,]] further comprising reacting the sample with a sulfonyl halide following reacting the sample with the sulfonhydrazide.

17. (Currently amended) The method of claim 16, wherein the sulfonyl halide ~~sulfonhydrazide~~ comprises



wherein X is Cl, Br, or I, and R is alkyl, substituted alkyl, aryl, or substituted aryl.

18. (Original) The method of claim 17, wherein R comprises lower alkyl.

19. (Original) A method for enhancing positive ion mode electrospray ionization efficiency of a carbonyl compound comprising reacting a carbonyl compound with a sulfonhydrazide to form a sulfonhydrazone of the carbonyl-containing compound that is efficiently ionized by electrospray ionization processes.

20. (Currently amended) The method of claim 19, wherein the carbonyl-containing compound is a ketosteroid.

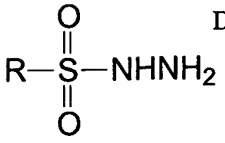
21. (Currently amended) The method of claim 20, wherein the ketosteroid is selected from the group consisting of androgens, corticoids, estrogens, sterols, vitamin D metabolites, phytosteroids, neurosteroids and bile acids, and combinations thereof.

22. (Currently amended) The method of claim 21, wherein the ketosteroid is an estrogen.

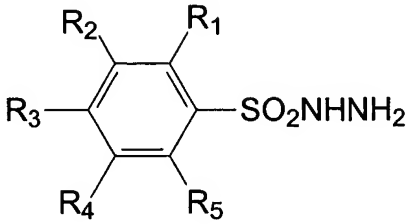
23. (Currently amended) The method of claim 22, wherein the estrogen is a catechol estrogen.

24. (Currently amended) The method of claim 19, wherein the sulfonhydrazide comprises

wherein R is selected from the group consisting of alkyl, substituted alkyl, aryl, and substituted aryl.



25. (Currently amended) The method of claim ~~19~~ 24, wherein the sulfonhydrazide comprises



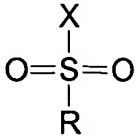
wherein R_1-R_5 are independently selected from the group consisting of hydrogen, C1-C5 alkyl, C1-C4 alkoxy, halogen, amino, nitro, hydroxyl, carbonyl, nitroso, cyano, and sulfonyl, and combinations thereof.

26. (Currently amended) The method of claim 25, wherein the sulfonhydrazide is *p*-toluenesulfonhydrazide.

27. (Currently amended) The method of claim 19[[,]] further comprising reacting the carbonyl compound with a sulfonyl halide after forming the sulfonylhydrazone.

28. (Original) The method of claim 27, wherein the sulfonyl halide comprises a sulfonyl chloride.

29. (Original) The method of claim 27, wherein the sulfonyl halide comprises



wherein X is Cl, Br, I, or any good leaving group, and R is alkyl, substituted alkyl, aryl, and substituted aryl.

30. (Original) A method for separating and detecting ketosteroids present in a biological sample, comprising:

extracting a ketosteroid from a biological sample to provide a concentrated sample of the ketosteroid;

reacting the concentrated sample of the ketosteroid with *p*-toluenesulfonhydrazide to form a *p*-toluenesulfonhydrazone derivative of the ketosteroid;

separating the *p*-toluenesulfonhydrazone derivative of the ketosteroid from other components in the concentrated sample by reverse phase liquid chromatography;

detecting the *p*-toluenesulfonhydrazone derivative of the ketosteroid by its API-MS signal to detect the ketosteroid in the sample.

31. (Currently amended) The method of claim 30[[,]] further comprising reacting the *p*-toluenesulfonhydrazone derivative of the ketosteroid with a sulfonyl halide to form a sulfonyl halide derivative of the *p*-toluenesulfonhydrazone derivative of the ketosteroid, prior to separating the *p*-toluenesulfonhydrazone derivative of the ketosteroid from other components.

32. (Original) The method of claim 30 further comprising adding a known amount of a deuterated analog of the ketosteroid to the biological sample prior to extracting to quantify the ketosteroid in the sample by comparison of API-MS signals from the ketosteroid and its deuterated analog.

33. (Currently amended) The method of claim 30, wherein the biological sample is urine.

34. (Currently amended) The method of claim 30, wherein the ketosteroid is an estrogen.

35. (Currently amended) The method of claim 34, wherein the estrogen is a catechol estrogen.

36. (Currently amended) The method of claim 30, wherein separating by liquid chromatography comprises separating by high performance liquid chromatography (HPLC).

37. (Currently amended) The method of claim 36, wherein separating by HPLC comprises separating by reverse phase HPLC in a methanol/water mobile phase and a C18 stationary phase.

38. (Original) A kit for use in a method for detecting a ketosteroid in a sample by MS, the kit comprising in packaged combination:

a sulfonhydrazide compound; and

a deuterated standard of the ketosteroid.

39. (Currently amended) The kit of claim 38[[,]] further comprising a sulfonyl halide.

40. (Currently amended) The kit of claim 38, wherein the sulfonhydrazide compound comprises *p*-toluenesulfonhydrazide.

41. (Original) The kit of claim 39, wherein the sulfonyl halide comprises sulfonyl chloride.

42. (Currently amended) The kit of claim 38, wherein the ketosteroid is a catechol estrogen and the deuterated standard is a deuterated catechol estrogen.

43. (Currently amended) A method for detecting an endogenous ~~keto~~steroid[[s]] in a sample, comprising:

reacting the sample with a carbonyl protecting reagent that reacts with a carbonyl group that may be present in the endogenous steroid ~~in the ketosteroid~~ to form a carbonyl derivative of the endogenous steroid, and then reacting the sample with a hydroxyl protecting reagent that reacts with a hydroxyl group present in the endogenous steroid to form a hydroxyl derivative, wherein both reacting steps together provide a derivatized endogenous steroid; and

analyzing the reacted sample by ~~ionization~~ mass spectrometry to detect the endogenous ~~keto~~steroid if it is present by detecting the derivatized endogenous steroid. ~~carbonyl derivative or the hydroxyl derivative of the ketosteroid.~~

44. (Currently amended) The method of claim 43[[,]] further comprising separating the endogenous ~~keto~~steroid from the reacted sample by liquid chromatography prior to analyzing the reacted sample.

45. (Currently amended) The method of claim 43, wherein the carbonyl protecting reagent comprises a compound[[s]] that forms an oxime derivative, a silyl derivative, an ketal/acetal, a hydrazone, or a Schiff's base derivative.

46. (Original) The method of claim 45, wherein the carbonyl protecting reagent comprises methoxyamine, ethoxyamine, carboxymethoxylamine, Girard's Reagent T, Giard's Reagent P, 6-ethoxy-2-benzothiazolesulfonamide, cystein, N'-(2-Thiazolyl) sulfanilamide, sulfisomidine, sulfadiazine, or p-toluenesulfohydrazide (TSH).

47. (Currently amended) The method of claim 46, wherein the hydroxyl protecting reagent comprises a compound that forms a silyl derivative, an acyl derivative, a benzoyl derivative, an alkyl derivative, a dansyl derivative, or a nitrobenzofurazan ~~nitrobenzofuran~~ derivative.

48. (Currently amended) The method of claim 47, wherein the hydroxyl protecting reagent comprises nitrobenzopentaflurobenzoyl hydroxylamine, hydroxylamine, dabsyl chloride, dansyl chloride, 1-fluoro-2,4-dinitrobenzene, or 4-fluoro-3-nitrobenzofurazan.

Please add the following new claims 49-51:

--49. (New) The method of claim 43, wherein the carbonyl protecting reagent comprises a sulfonhydrazide.

50. (New) The method of claim 43, wherein the hydroxyl protecting reagent comprises a sulfonyl halide.

51. (New) The method of claim 43, wherein the carbonyl protecting reagent comprises a sulfonhydrazide and the hydroxyl protecting reagent comprises a sulfonyl halide.--